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Research Article

Study of Phytochemical Screening, Physicochemical Analysis and Antimicrobial Activity of *Bacopa monnieri* (L) Extracts

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ABSTRACT

Bacopa monnieri (L) popularly known as Brahmi is an important nervine herb in an ayurvedic medicine. It belongs to the family Scrophulariaceae. It has been traditionally used as ethno medicine and is useful to treat anxiety, anger, insomnia, nerve pain, concentration difficulties and learning problems. It has also been used as a cardio tonic, digestive aid and improves respiratory function. It shows antioxidant, antiaging, antidepressant, anticancer and antibacterial activity. The present study was carried out to determine the phytochemical constituents and physicochemical values according to the pharmacopoeial method. The antimicrobial activity of *Bacopa monnieri* (L) was also investigated by using aqueous and methanolic extracts against two gram positive, two gram negative bacteria and two fungal organisms at 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml concentrations. The methanolic extractive value of *Bacopa monnieri* (L) (10.1%) was found highest followed by ethanol (8.6%), aqueous (7.6%), chloroform (2%), acetone (1.5%), dichloromethane (0.6%), ethyl acetate (0.5%) and petroleum ether (0.5%) extract. Phytochemical investigation of *Bacopa monnieri* (L) revealed the presence of various important secondary metabolites such as carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins in methanolic, ethanolic and aqueous extracts. No activity was observed against bacterial strains like *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* when subjected to aqueous and methanolic extract of *Bacopa monnieri* (L). Methanolic extract showed significant antifungal activity against *Candida albicans* and *Aspergillus niger* at 2.5mg/ml and 1.25mg/ml concentrations.

Keywords: *Bacopa monnieri* (Linn), Extractive value, Physicochemical, Antidepressant, Antimicrobial.

INTRODUCTION

According to World Health Organization (WHO) majority of the world's population use traditional medicines for their primary health care needs. Medicinal plants are the most important natural resource of life saving drugs. Secondary metabolites of plants possesses biological properties such as antioxidant, antiapoptosis, antiaging, anticarcinogen, antiinflammatory, antiatherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation activity[1-7]. *Bacopa monnieri* (L) belongs to the family Scrophulariaceae, is a creeping, glabrous, succulent herb grows in marshy areas throughout India. It has been traditionally used to treat anxiety, anger, nerve pain, insomnia, learning problems and concentration difficulties[8,9]. It has been reported that it is used in the treatment of epilepsy and asthma[10]. It has also been used as a cardio tonic, digestive aid and to improve respiratory function[11]. Natural antioxidants such as flavonoids, tannins and phenols are increasingly attracting because they are disease preventing, health promoting and antiaging substances[12]. It was reported that antioxidant properties of *Bacopa monnieri* (L) offer the protection from free radical damage in cardiovascular diseases, certain types of cancer and helps to prevent induced lipid peroxidation[3]. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening is an important tool in bioactive compound analysis[13]. Identification, separation, quantification and standardization of major phytochemical compounds were carried out by many researchers using advance techniques[14-16]. In India, infectious diseases account for high proportions of health problems. Infections are due to variety of bacterial etiologic agents, such as pathogenic *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shingella dysenteriae* are most common[17]. Considering the increased incidence of severe fungal and bacterial infections in immunologically deficient patients, there is a great need in finding new classes of natural products that may be effective against antibiotic resistant bacteria and fungi. Therefore, the objective of the present study was to determine the effective solvent for extraction of *Bacopa monnieri* (L), to determine the ash value, to study the phytochemical screening in methanolic, ethanolic and aqueous extracts of *Bacopa monnieri* (L) and to investigate the antimicrobial activity of *Bacopa monnieri* (L) extracts in order to use it as a possible source for new antimicrobial substances against human pathogens.

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Table 1: Extractive value (%) of *Bacopa monnieri* (L).

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Methanol	2	Yellowish green	10.1
Ethanol	2	Green	8.6
Aqueous	2	Dark brown	7.6
Chloroform	2	Light green	2
Acetone	2	Green	1.5
Dichloromethane	2	Light green	0.6
Ethyl acetate	2	Green	0.5
Petroleum Ether	2	Colorless	0.5

Table 2: Ash value and loss on drying of *Bacopa monnieri* (L).

Total ash value	13.5%
Acid insoluble ash value	5.5%
Water soluble ash value	2.5%
Loss on Drying	1.5%

MATERIAL AND METHODS

Plant material

The herbarium of *Bacopa monnieri* (L) was prepared and authentication has been obtained from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The specimen (MGJ-1) was deposited to herbarium department in Botanical Survey of India, Pune. The whole *Bacopa monnieri* (L) plant material was shade dried at room temperature and kept in oven at 40ºC to remove moisture. The dried plant was then finely ground by mechanical grinder. The powder obtained was then sieved and kept in air tight containers for further extraction process.

METHODS

Extraction of Bacopa monnieri (L)

The extracts of *Bacopa monnieri* (L) were prepared by traditional maceration method. *Bacopa monnieri* (L) dry plant powder was dissolved in methanol, ethanol and water. Each mixture was kept separately for 72 hrs at room temperature with occasional shaking. After completion of the maceration, the supernatant was decanted and the mixture was filtered. The extract was concentrated to dryness by keeping filtrate for complete evaporation of the solvent. After evaporation of solvent the extract was weighed and kept in air tight glass container for the determination of phytochemical constituents and antimicrobial activity. Aqueous extract was reconstituted in sterile distilled water with the required concentration for antimicrobial activity. Methanolic extracted powder was suspended in absolute methanol to prepare the desired concentration of extract solution. Four concentration stocks 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml were prepared and used for antimicrobial activity.

Physicochemical Analysis[18]

Determination of Extractive value of Bacopa monnieri (L)

The dry powdered plant material of *Bacopa monnieri* (L) was extracted with water, methanol, ethanol, acetone, chloroform, dichloromethane, ethyl acetate and petroleum ether using a maceration process. The coarsely powdered plant material was weighed and transferred into a dry conical flask. Then each flask was filled with different solvents separately. The flasks were corked and kept aside for 24 hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. 1 filter paper. After the filtrate has obtained, it was then transferred into a weighed petry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent.

The extractive value in percentage was calculated by using following formula.

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Determination of Ash value of Bacopa monnieri (L)

Total ash

Two gram coarsely powdered dry plant material of *Bacopa monnieri* (L) was weighed in a previously ignited crucible and ignited gradually by heating to 500-600ºC until it become white. Cooled in desiccator and weighed. The content of total ash in terms of percentage was calculated.

Acid-Insoluble Ash

It is residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. 25ml of dilute HCl was added to the crucible containing total ash and boiled gently for 5min. The insoluble matter was collected on ashless filter paper, washed with hot water then filter paper was ignited, cooled in desiccator and weighed. The content of acid insoluble ash in terms of percentage was calculated.

Water Soluble Ash Values

The total ash was boiled for five minutes with 25 ml of water, the soluble matter was collected in a crucible, ignited and weighed. The content of water soluble ash in terms of percentage was calculated.

Loss on Drying

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 2 g of *Bacopa monnieri* (L) powder was taken in glass petri dish. The Petri dish was kept open in vacuum oven and dried at a temperature between 100 to 105°C for 2 h until a constant weight is recorded. Then cooled in a desiccator to room temperature, weighed and recorded. Percent loss on drying was calculated using the following formula.

Table 3: Phytochemical screening of aqueous, methanolic and ethanolic extracts of *Bacopa monnieri* (L).

S. No.	Secondary metabolites	Phytochemical tests	Methanol	Ethanol	Aqueous
1.	Carbohydrates	Molisch's Test	+	+	+
2.	Proteins	Millon's Reagent Test	+	+	+
3.	Amino acid	Ninhydrin Test	-	-	+
4.	Steriod	Liebermann Burchard Reaction	+	+	+
5.	Glycosides	Legal's Test	+	+	+
	a)Cardiac glycosides				
	b)Anthraquinone glycosides	Borntrager's Test	-	-	-
	c)Saponin glycosides	Foam Test	+	+	+
6.	Flavonoids	Sodium hydroxide test	+	+	+
7.	Alkaloids	Mayer's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
8.	Tannins	Dilute Nitric acid Test	+	+	+

+ Present, - Absent.

Loss on drying (%) =

$$\frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

In the present investigation the highest extractive value was observed in methanolic extract followed by ethanol and aqueous extract of *Bacopa monnieri* (L), therefore these extracts were subjected to further phytochemical screening.

Phytochemical screening of methanolic, ethanolic and aqueous extracts of Bacopa monnieri (L)[18]

The different phytochemical tests were performed for establishing the profile of plant extract for its phytochemical constituents. The phytochemical screening of *Bacopa monnieri* (L) for Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Cardiac glycosides, Anthraquinone glycosides, Saponin glycosides, Flavonoids, Alkaloids and Tannins was carried out. The extract obtained from methanol, ethanol and aqueous was used for phytochemical screening.

Test for carbohydrates

Molisch's test

3ml of extract was taken in a test tube, 2 drops of alcoholic alpha naphthol solution was added, shaked well and then 1ml of concentrated sulfuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carohydrates.

Test for proteins

Millon's test

About 3ml of sample extract was treated with 5ml of Million's reagent. White pricipitate was obtained. The mixture was then warmed, pricipitate turned to brick red. It indicated the presence of proteins.

Test for amino acids

Ninhydrin test

About 3ml of plant extract solution was heated followed by addition of 3 drops of 5% ninhydrin solution. The test tubes with this solution were kept in boiling waterbath for 10 minutes. The purple color was observed. It indicated the presence of amino acids.

Test for steroids

Leibermann- Burchard reaction–

To 3ml extract 10ml chloroform was added followed by 2ml of acetic anhydride. Then 2 drops of concentrated sulfuric acid were added from the side of the test tube. The blue green color appearance indicated the presence of steroids.

Test for glycosides

Cardiac Glycosides (Legal's Test)

To the 3ml extract 1ml pyridine was added by frequent shaking followed by 1ml sodium nitroprusside. Pink to red color appeared. It indicated the presence of cardiac glycosides.

Anthraquinone glycosides (Borntrager's test)

To 3ml extract dil. Sulfuric acid was added. The solution was then filtered. Then equal volume of chloroform was added to the filtrate. After shaking organic solvent was separated. Finally equal volume of ammonia solution was added. No bright pink, red or violet color was developed in the upper layer which indicated the absence of anthraquinones.

Saponin glycosides (Foam test)

About 50mg of extract was diluted in the successive solvents and made up to 20ml. The suspension was shaken for 15min. A 2cm layer of foam appeared. Appearance of foam indicated the presence of saponins.

Test for flavonoids

Sodium hydroxide test

To 3ml of extract increasing amount of sodium hydroxide was added it showed colouration, which was decolorised after addition of dil. hydrochloric acid. Decolorization showed presence of flavonoids.

Test for alkaloids

Solvent free extract was stirred with 10ml of dilute hydrochloric acid and filtered. The filtrate was tested with following alkaloidal reagents as follows:

Mayer's test

To 3ml filtrate two drops of Mayer's reagent were added by the sides of the test tube. A white creamy precipitate was observed. It indicated positive test.

Hager's test

To 3ml of filtrate, 1ml of Hager's reagent was added. A prominent yellow precipitate occurred. It indicated the presence of alkaloids.

Wagner's test

Table 4: Labels and each well used during the experiment for antimicrobial activity *Bacopa monnieri* (L).

S. No.	Plate Label	Extract used	Test Organism	Well No.	Concentration of extract
1.	SF1	Aqueous	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
2.	SF1	Aqueous	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
3.	SF2	Methanolic	*Staphylococcus aureus*	1,2,3	10mg/ml,5mg/ml, control respectively
4.	SF2	Methanolic	*Staphylococcus aureus*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
5.	ST1	Aqueous	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
6.	ST1	Aqueous	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
7.	ST2	Methanolic	*Bacillus subtilis*	1,2,3	10mg/ml,5mg/ml, control respectively
8.	ST2	Methanolic	*Bacillus subtilis*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
9.	EC1	Aqueous	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
10.	EC1	Aqueous	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
11.	EC2	Methanolic	*Escherichia coli*	1,2,3	10mg/ml,5mg/ml, control respectively
12.	EC2	Methanolic	*Escherichia coli*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
13.	P1	Aqueous	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
14.	P1	Aqueous	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
15.	P2	Methanolic	*Pseudomonas aeruginosa*	1,2,3	10mg/ml,5mg/ml, control respectively
16.	P2	Methanolic	*Pseudomonas aeruginosa*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
17.	A1	Aqueous	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
18.	A1	Aqueous	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
19.	A2	Methanolic	*Aspergillus niger*	1,2,3	10mg/ml,5mg/ml, control respectively
20.	A2	Methanolic	*Aspergillus niger*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
21.	C1	Aqueous	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
22.	C1	Aqueous	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively
23.	C2	Methanolic	*Candida albicans*	1,2,3	10mg/ml,5mg/ml, control respectively
24.	C2	Methanolic	*Candida albicans*	4,5,6	2.5mg/ml,1.25mg/ml, control respectively

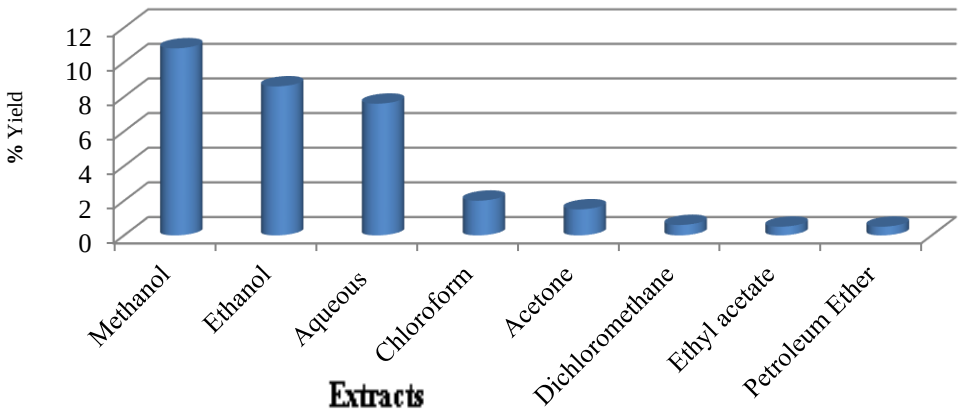

Figure 1: Extractive value (%) different extracts of *Bacopa monnieri* (L).

To 3ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the presence of alkaloids.

Test for Tannins

Nitric acid test

3ml of extract was taken in test tube, a few drops of dilute nitric acid were added. The reddish yellow color indicated the presence of tannins.

Investigation of antimicrobial activity

Microorganism strains

Four bacterial and two fungal test organisms were selected for this activity. Identification of the isolates was established by 16S rDNA and Internal Transcribed Spacer (ITS) sequencing respectively. Following strains of microorganisms were used for antimicrobial activity.

1. *Staphylococus aureus* (Gram +ve)
2. *Bacillus subtilis* (Gram +ve)
3. *Escherichia coli* (Gram -ve)
4. *Pseudomonas aeruginosa* (Gram -ve)
5. *Aspergillus niger*
6. *Candida albicans*

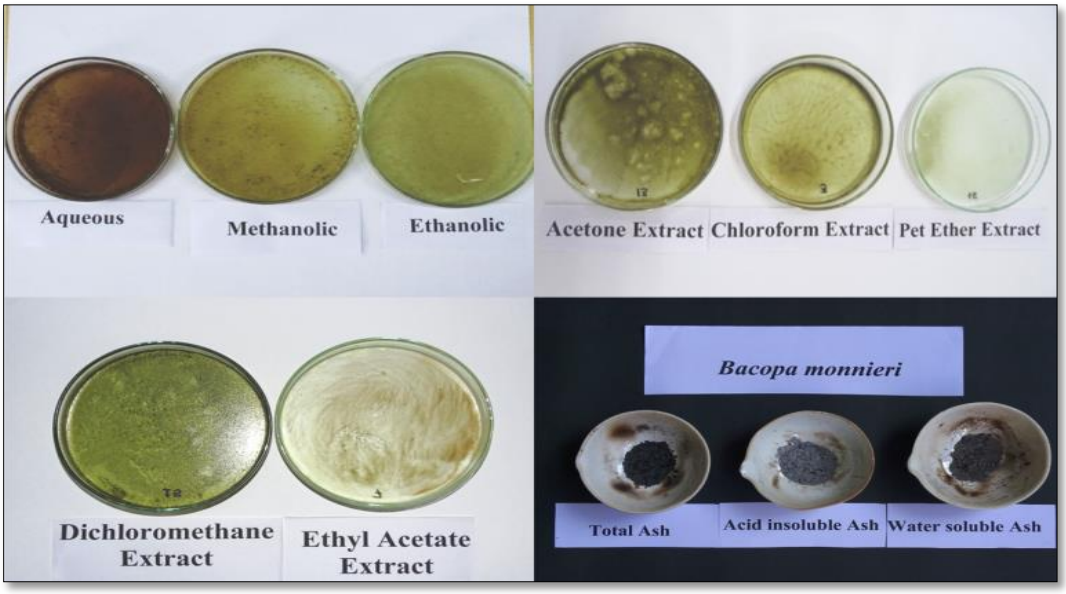

Plate No. 1: Aqueous, methanolic, ethanolic, acetone, chloroform, petroleum ether, dichloromethane and ethyl acetate extract of *Bacopa monnieri* (L).

Screening for antimicrobial activity of Bacopa monnieri (L)

Antimicrobial activity was performed by the agar well diffusion method. In this method, pure isolate of each microbe was sub cultured on the nutrient agar media plates at 37ºC for 24 h. For fungal cultures YPD agar medium was used. One plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 cfu/ml (standardized by 0.5 McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microlitre (100µl) of inoculum of each test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 10 mm were made with a sterile borer in the inoculated agar. A 100µl volume of each extract was propelled directly into the wells of the inoculated agar plates for each test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37ºC for 24h. Sterile water and methanol was used as negative control in each respective plate for analysis. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 10 mm.

RESULTS

Following are the labels and each well used during the experiment for antimicrobial activity of *Bacopa monnieri* (L).

Well No 3 and 6 are control in each plate, Well No.1: 10 mg/ml, Well No.2: 5 mg/ml, Well No.4: 2.5mg/ml, Well No.5: 1.25mg/ml

RESULTS AND DISCUSSION

In the present study the extractive value of *Bacopa monnieri* (L) in methanol, ethanol, aqueous, chloroform, acetone, dichloromethane, ethyl acetate and petroleum ether extract was determined. The extractive value and color of extracts of *Bacopa monnieri* (L) was investigated and represented in Table No. 1. From the present study it was found that, the extractive value of *Bacopa monnieri* (L) in methanolic extract was maximum (10.1%) as compared to other extracts. The ethanolic extract showed slightly less extractive value (8.6%) than methanolic extract of *Bacopa monnieri* (L). The extractive value of *Bacopa monnieri* (L) in aqueous extract was 7.6% followed by chloroform extract (2%), acetone extract (1.5%) and dichloromethane (0.6%). The ethyl acetate and petroleum ether extract showed very less (0.5 %) extractive value. The color of extracts observed was yellowish green in methanol, green in ethanol, acetone and ethyl acetate extract, dark brown in aqueous, light green in chloroform, dichloromethane and colorless in petroleum ether extract (Table 1). From the literature review it was observed that more yield percentage of *Bacopa monnieri* (L) was obtained in alcoholic extract[19]. The total ash, acid insoluble, water soluble ash value and loss on drying are depicted in Table No. 2. The physicochemical analysis of *Bacopa monnieri* (L) are presented in Plate No. 1. Plant synthesises a broad range of primary and secondary metabolites with different functional groups[20]. Phytochemical screening is an important tool in bioactive compound analysis. It is quick, inexpensive and simple procedure that shows the various types of phytochemicals present in plant. The presence of phytochemicals is a marker that the plant can be a prospective source of precursors in the formation of synthetic drug[21]. It was reported that carbohydrates, phenols glycosides and anthraquinones were present in petroleum ether and ethanolic extract of *Bacopa monnieri* (L)[22]. The in vitro phytochemical analysis of roots of *Bacopa monnieri* (L)

Table 5: Antibacterial and antifungal activity of aqueous and methanolic extract of *Bacopa monnieri* (L).

Diameter of Growth Inhibition Zone (mm)						
Extract and its concentration	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
Aqueous extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
5 mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
2.5mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
1.25mg/ml	*No*	*No*	*No*	*No*	*No*	*No*
Methanolic extract						
10mg/ml	*No*	*No*	*No*	*No*	*No*	23
5 mg/ml	*No*	*No*	*No*	*No*	*No*	23
2.5mg/ml	*No*	*No*	*No*	*No*	35	22
1.25mg/ml	*No*	*No*	*No*	*No*	25	22
Control	*No*	*No*	*No*	*No*	25	20

Note: Values including diameter of wells.
No: No inhibition was observed.

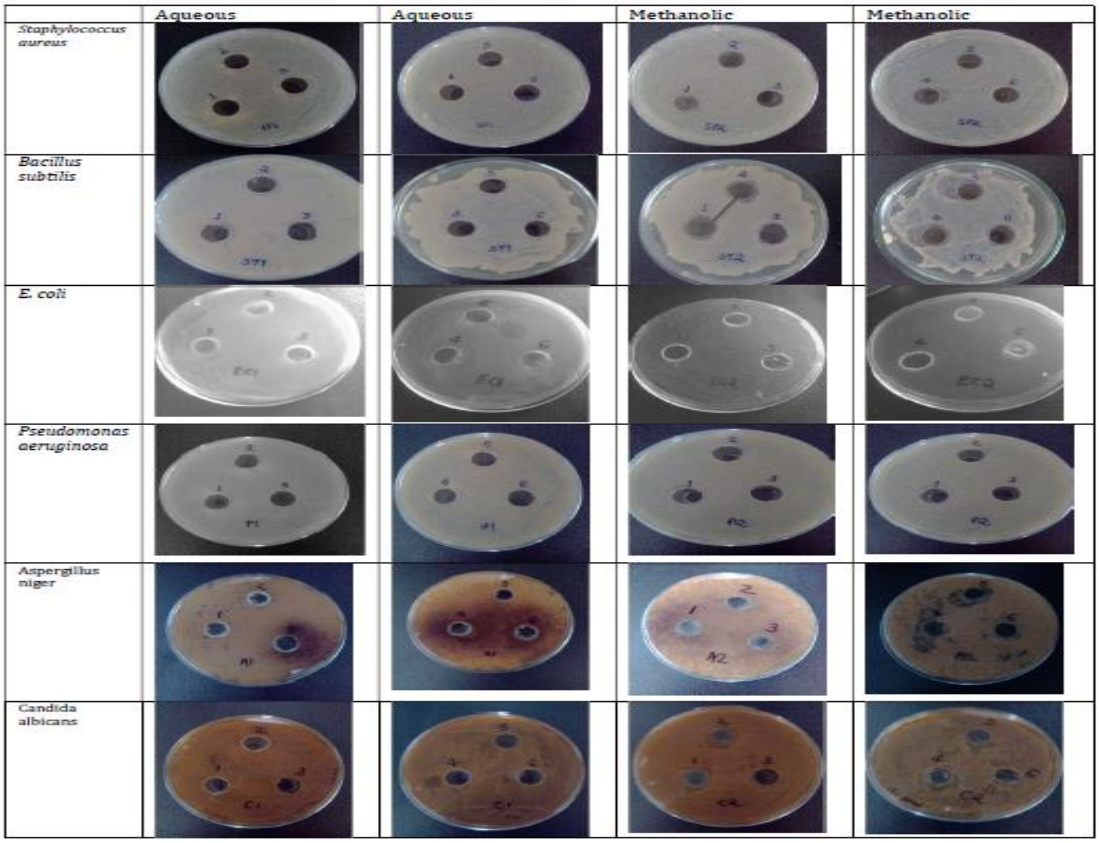

Plate No. 2: Antimicrobial activity of *Bacopa monnieri* (L) in aqueous and methanolic extract.

was carried out, the study showed presence of alkaloids, anthraquinone, cardiac glycosides, flavanoids, phenols, saponin, steroids, tannins, terpenoids, alkaloids in ethanolic, methanolic, chloroform, petroleum ether and ethyl acetate extract[17]. *Bacopa monnieri* (L) was reported to possess terpenoids and steroids predominately in ethanol, aqueous, chloroform, acetone and ethyl acetate extracts[23]. The aqueous and hydroalcoholic extracts of *Bacopa monnieri* (L) were reported for the presence of phenols, flavanoids, glycosides, alkaloids and carbohydrates[24]. The phytochemical analysis of leaf callus of *Bacopa monnieri* (L) was carried out by Singh, he observed the presence of tannins, saponins, terpenoids, steroids in ethanol and aqueous extract and absence of anthraquinone glycosides and phenols in same extracts[25]. From the present study phytochemical screening revealed that saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids were present in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L). The aqueous extract of *Bacopa monnieri* (L) showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids. Anthraquinone glycosides were absent in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* (L). (Table No. 3). The antimicrobial activity study revealed that, the pattern of inhibition varied with the plant extract

and the organism tested. The highest antifungal activity was observed in methanolic extract and maximum zone of inhibition was observed against *Aspergillus niger* and *Candida albicans*, where as in aqueous extract no antifungal activity was observed. The zone of inhibition of methanolic extract was highest for *Candida albicans* at 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml concentrations, while for *Aspergillus niger* the highest zone of inhibition was observed at 2.5mg/ml and 1.25mg/ml concentrations. No antibacterial activity was observed against *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* in aqueous and methanolic extracts of *Bacopa monnieri* (L) in these used concentrations. Antimicrobial activity of *Bacopa monnieri* (L) aqueous and methanolic extract are presented in Table No. 5. and Plate No. 2. Earlier studies have reported that, methanolic extracts of *Bacopa monnieri* (L) was found to possess maximum inhibitory effects against gram positive and gram negative organisms tested compared to chloroform and ethanolic extract[26]. Therapeutic value of medicinal plants and bioactivity of extract lies in the various phytochemicals present in it, plant rich in tannins have antimicrobial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane[27]. Flavonoids are the major group of phenolic compounds reported to have antimicrobial activity[28]. The extracts of seeds of *Vitexagnus-castus* was reported to possess antimicrobial activity which is associated with its alkaloid, saponin, taninn, flavonoid and glycoside contents[29]. Phenolic compounds such as coumarin and quercetin had extended protection to gastroenteritis disease causing microbes[30].The antimicrobial activity of *Bacopa monnieri* (L) extract as recorded in the present study may, therefore, be attributed to the phytoconstituents present in it.

CONCLUSION

From the present study it can be concluded that, the extractive value is useful to find the effective solvent for extraction process. It gives idea about the nature of phytochemical constituents present in the plant material. In the present study methanol has been found to be pre-eminent solvent used for extraction. The ash value helps to determine purity of a crude plant material and foreign inorganic matter present as an impurity. For further analytical study of *Bacopa monnieri* (L) ash value is useful as it removes all traces of organic matter which may interfere further experimentation. Loss on drying test is effective to measure the amount of moisture content and volatile matters in a sample. Higher water content may prone to have chemical and microbial decomposition of crude drug. The phytochemical screening is helpful for confirmation of bioactive phytochemical constituents in *Bacopa monnieri* (L). The loss on drying has been found less in *Bacopa monnieri* (L). No inhibitory effect was observed against bacterial strains like *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* when subjected to aqueous and methanolic extract of *Bacopa monnieri* (L) with four concentration stocks used for this study perhaps it may show antibacterial activity in some other concentrations which need further investigation.The promising anti fungal activity of *Bacopa monnieri* (L) may be due to presence of phytochemicals such as alkaloids, phytosterols, proteins, Flavonoids, tannins etc.. which will be helpful in future to treat certain fungal diseases and will be used in therapeutic natural drugs.

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